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High-resolution two-dimensional protein mapping. Technique and applications, with special reference to the characterization of human lymphomas.

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Summary.

The major objective of the work described in this thesis was to investigate, whether biochemical 'multi-protein analysis' (see Chapter 1) of normal and tumor tissues can contribute to the problems of tumor characterization and classification. High-resolution two-dimensional electrophoresis, known as 'protein mapping' (i.e. separation of proteins by isoelectric focusing [IEF] in the first dimension followed by SDS-electrophoresis in the second dimension), has been applied to separate membrane proteins isolated from normal human lymph nodes and those of lymphoma patients. Special attention was paid to centroblastic/centrocytic lymphoma (CB/CC) and lymphogranulomatosis (LGR, Hodgkin's disease), due to the relatively high incidence rates of these tumors. The technical/analytical aspects of the method have been treated in Chapter 2, some general features of the obtained maps in Chapter 3.

A special problem was presented by the comparison of the complex 2D patterns (Chapter 4). Two different methods were applied. The results of the first method - a purely manual procedure, which takes the morphological information as the starting point (section 4.1) - can be summarized as follows: 1) Proteins were found, the presence or absence of which corresponded with the morphological diagnosis. 2) Screening for the presence of these marker proteins allowed the identification of maps of 'unknown' lymph nodes with reasonable certainty.

In the second method, in which the protein maps were treated as 'multi-parameter systems' and the protein spots as the 'characters' of the sample, two techniques of numerical taxonomy were applied, i.e. cluster analysis and multi-dimensional scaling (section 4.2). The results of this approach can be summarized as follows: The maps as such could not be classified into the three groups corresponding with the three diagnoses normal, CB/CC, and LGR. However, by taking into account the morphological information, biochemical subtypes of tumors with the same

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diagnosis could be recognized, both for CB/CC and LGR.

Suggestions for improvement, notably on automation, have been made in Chapter 5.

In the Appendices, besides reports on the work summarized above (A-C), some aspects of the methods have been investigated by the analysis of systems of less complexity than that of the human lymphomas, i.e. of water-soluble proteins from the germ leaves of four closely related plant species (Appendix D), and by the application of multi-dimensional scaling to amino acid sequence data of mammalian ribonucleases (Appendix E).